The contribution of insect prey to the total nitrogen content of sundews (*Drosera* spp.) determined *in situ* by stable isotope analysis

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Summary

- The contribution of insect prey to total N in the carnivorous plants, *Drosera rotundifolia* and *D. intermedia*, was quantified *in situ* and without any experimental manipulation using natural abundance stable isotope analysis.
- Samples of *D. rotundifolia* and *D. intermedia*, insects and noncarnivorous reference plants were collected from three contrasting locations across Britain. The proportion of *Drosera* nitrogen obtained from insect prey was calculated by a mixing model using $\delta^{15}N$ values from the different plant groups.
- The mean proportion of *Drosera* N derived from prey was 50%. There were significant differences in this proportion between sites, and significant differences within sites. There were significant differences between plant tissues and a significant negative relationship between the proportion of N derived from prey and the C : N ratio of *Drosera* tissues.
- There was little evidence of differences in prey capture/utilisation in response to N availability, possibly due to a limited range in available N between the sites. However, evidence of a positive benefit of prey capture was apparent through the decrease in C : N ratio with increasing prey N concentrations in the plants.

Key words: sundews, *Drosera rotundifolia*, *Drosera intermedia*, stable isotope analysis, carnivorous plants, nitrogen, carbon.

Introduction

The ability of carnivorous plants to capture and digest animal prey has been observed in many studies (e.g. Darwin, 1875; Karlsson *et al.*, 1987; Schulze *et al.*, 1997). Indeed, this carnivorous habit has been described in over 600 different species (Adamec, 1997). Insect capture has been assumed to supplement root nutrient uptake, with emphasis on the acquisition of additional N from the captured prey, and this is presumed to be an adaptation to a low nutrient environment (e.g. Givinish *et al.*, 1984). Ellison & Gotelli (2001) have recently reviewed the evolutionary ecology of carnivory in plants.

Carnivorous plants have been shown to benefit from insect capture through increased growth, earlier flowering and increased seed production. Darwin (1878) observed increased flower and seed production in *Drosera rotundifolia* when feeding rates were artificially increased. Thum (1988), Aldenius *et al.* (1983) and Thoren & Karlsson (1998) also observed an increase in growth parameters, in response to increased prey capture, in *Drosera* species. Thoren & Karlsson (1998) concluded that the growth of the carnivorous plants they studied was limited by prey capture. However, such positive effects of prey capture have not always been observed. When insects were excluded from *D. rotundifolia* in the field, Stewart & Nilsen (1991) observed no difference in growth or flowering

between plants that caught no prey and those that were allowed to continue trapping prey. Similarly, *Utricularia* species grown in the laboratory with no access to insects were able to flower and grow to maturity (Dore Swamy & Mohan Ram, 1971), showing that insect capture is not a requirement for normal growth and development.

Studies on the likely benefit of carnivory to plants have emphasised the role of N uptake from the prey. Chandler & Anderson (1976), and Dixon *et al.* (1980) measured plant uptake of the total N contained in their insect prey to be 29% and 76%, respectively. Indirect measurements of the contribution of prey N to carnivorous plants, using ^{15}N-labelled prey, yielded estimates of 2%–28% of the plant total N turnover (Pate & Dixon, 1978; Hanslin & Karlsson, 1996). However, these measurements involved adding prey to the plants, possibly altering both the rate of prey capture and the uptake of nutrients from captured prey. Methods that involve no manipulation of plant N nutrition should provide more realistic estimates of the true *in situ* contribution of insect prey to the N nutrition of carnivorous plants.

Natural abundance stable isotope measurements have been used to calculate directly the contribution of two N sources to a single N sink (Peterson & Fry, 1987; Treseder *et al.*, 1995). As carnivorous plants may have two sources of nitrogen (insect-derived and root-derived) this method has been used to quantify the contribution of insect nitrogen to total plant N content. Schulze *et al.* (1991) found that prey-derived nitrogen contributed between 0% and 55% of the total nitrogen content of various Australian *Drosera* species. Values for the tropical pitcher plants *Nepenthes rafflesiana* and *N. albomarginata* were 53% and 68%, respectively (Moran *et al.*, 2001).

The wide variation in the relative contribution of insect-derived N to total plant N content indicates that an external variable may affect the contribution of prey N to the plants. The cost-benefit model derived by Givinish *et al.* (1984) predicts that carnivorous plants are better suited to low-nutrient environments and that the significance of carnivory will reduce with increasing nutrient levels. The adaptation of this model by Karlsson *et al.* (1991) to relative growth rates suggests that the impact of prey capture on growth and survival will not change with increasing nutrient level; rather it is the relative decrease in competitive ability that precludes carnivorous plants from more nutrient rich sites.

The primary aim of this study was to use natural abundance stable isotope analyses to quantify the relative contribution of insect-derived N to total plant N in the carnivorous plant *D. rotundifolia*, at three contrasting sites in Great Britain. We hypothesised first that the proportion of N obtained from prey would be higher at sites with lower inorganic N availability and second that under constant conditions of inorganic nitrogen availability, an increased proportion of N obtained from prey should relieve nitrogen limitation, which would be reflected by lower C : N ratios in plants.

Materials and Methods

Site descriptions and sampling methods

Plants were collected from three study sites in the United Kingdom. (1) Red Hill Bog (Ordnance Survey grid reference SU266017; latitude N50 : 48 : 51; longitude W1 : 37 : 26) is a valley mire in the New Forest National Park in southern England, dominated by *Sphagnum* species. The mean annual temperature is 9–10°C and mean annual precipitation is less than 700 mm. (2) The study site in the Lake District National Park (Ordnance Survey grid reference NY332057; latitude N54 : 26 : 32; longitude W3 : 01 : 53) is a *Sphagnum*-dominated blanket mire in the north-west of England. Mean annual temperature for the area is 6–7°C and mean annual precipitation is greater than 2300 mm. (3) The Rannoch Moor study site (Ordnance Survey grid reference NN249548; latitude N56 : 39 : 08; longitude W4 : 51 : 28) is located in the western highlands of Scotland. Here plants were removed from *Sphagnum*-dominated bog pools. The mean annual temperature for the area is less than 4°C and the mean annual precipitation is greater than 2300 mm. Samples were collected from the three sites on the following dates: New Forest 12–15 July 2001; Rannoch Moor 21–4 July 2001 and The Lake District 27–28 July 2001.

At each site, five 1 × 2-m plots were randomly allocated. This was done at the New Forest and Lake District sites by using random coordinates to select points on an imaginary grid; because the distribution of *Drosera* at the Rannoch Moor site was more discrete, due to the plants occurring mainly within the bog pools, a similar method was used, but only including the bog pools in the grid. From each plot five *D. rotundifolia* plants were removed; this was also done using random coordinates on an imaginary grid. Seven extra *D. rotundifolia* plants were removed from plot four in the Lake District and separated into leaves, flowers and below-ground organs ('roots'). In addition, five *D. intermedia* plants were removed from two plots at the Rannoch Moor site, and from two plots at the New Forest site; this species was less abundant than *D. rotundifolia* at these two sites and was not found at the Lake District site.

Two separate samples of noncarnivorous plants were also taken from each plot. One aggregated sample of *Sphagnum* spp. was created by collecting approximately 5 cm^3 of the closest *Sphagnum* to each *Drosera* plant removed (this was often the *Sphagnum* in which the *Drosera* plant was growing). One sample representative of the noncarnivorous vascular plants (NCVP) in the plot was removed. This involved removing whole plants from next to each *Drosera* plant, including one of each species present. The species used in the NCVP sample were very similar for all sites; the main species removed were *Erica tetralix*, *Carex* spp., *Eriophorum angustifolium*, *Molinia caerulea*, *Juncus* spp., and *Salix repens*. The *Sphagnum* spp. were used for direct comparison with

the *Drosera* plants because their shallow rooting depth was most similar to that of the *Drosera* plants. Also the NCVPs contained more woody tissues with higher C : N ratios, which may have affected their overall $\delta^{15}N$ values, resulting in an erroneous comparison. For all plants care was taken to remove all above- and below-ground plant tissues.

Insect samples to represent potential prey were taken from each plot using polystyrol glue traps (20 × 7 cm) covered with a clear, nondrying glue (Deathtrap Whitefly Catcher, Gerhardt Pharmaceuticals Ltd) placed in the centre of the plot for 24 h. Samples sizes were approximately 20 insects per trap. A second sample of actual insect prey was taken directly by removing trapped insects from the leaves of *Drosera* plants. Heavy rain at the New Forest and Rannoch Moor sites resulted in only a small insect sample being obtained (approximately 2–5 insects). Only one sample (approximately 20 insects) was taken from the Lake District site due to its small size. The insect samples from both the traps and the *Drosera* plants were from the order Diptera (flies). As the insects captured were of the same order and no significant difference was found between $\delta^{15}N$ of the insects that were trapped and those collected from *Drosera* plants ($P = 0.208$), for subsequent comparison of insect isotope values with those of *Drosera* all insect values from each site were pooled.

Sample preparation and analysis

Plant material was washed with deionised water. Plant and insect material was dried at 65°C for 72 h. *Drosera* and insect samples were ground to a fine powder using a pestle and mortar. *Sphagnum* and NCVP samples were ground under liquid nitrogen in a freezer mill (Spex CertiPrep). Insect and plant samples were analysed for $\delta^{15}N$ and $\delta^{13}C$ using a Carlo Erba C/N/S analyser interfaced with a Finnigan Tracer Mass Isotope Ratio Mass Spectrometer. Results are given using the δ notation expressed in units of per mil (‰) where $\delta = [(R_{sample}/R_{reference}) - 1] \times 1000$, and $R = {}^{13}C : {}^{12}C$ or ${}^{15}N : {}^{14}N$. All data are reported with respect to the following international standards: V-Pee Dee Belemnite for $\delta^{13}C$ and nitrogen in air for $\delta^{15}N$. Precision was 0.2‰ for $\delta^{13}C$ and 0.3‰ for $\delta^{15}N$.

The sites were very wet and there was no discernable substrate into which the plants were rooted. Therefore, it is likely that the plants would have obtained a large proportion of their nutrients from the water at the sites. Water samples were collected from each site by squeezing water from the wet peat/*Sphagnum* in which the *Drosera* plants were growing. The samples were filtered through Whatman GF/C glass fibre filters and frozen until required for analysis. Samples were later analysed for ammonia ($NH_3 + NH_4^+ - N$) and nitrate + nitrite using standard colorimetric methods (Mackereth *et al.*, 1989). Together these results gave total dissolved inorganic nitrogen (DIN) concentrations.

Data analysis

The relative contribution of insect N to the N content of the *Drosera* plants (%N) was calculated using the approach of Moran *et al.* (2001):

$$\%N \text{ from insect prey} = \frac{\delta^{15}N_A - \delta^{15}N_B}{\delta^{15}N_C - \delta^{15}N_B}$$

where $\delta^{15}N_A$ is $\delta^{15}N$ of the insectivorous plant, $\delta^{15}N_B$ is $\delta^{15}N$ of the *Sphagnum* spp. from the corresponding plot and $\delta^{15}N_C$ is the mean $\delta^{15}N$ of all the insects from the site. The limitations of the mixing model used have been discussed by Moran *et al.* (2001). The assumptions of the model are (a) that base line resources for both types are similar in nitrogen isotopic composition, and (b) $\delta^{15}N$ of noncarnivorous plants can be used as a surrogate for $\delta^{15}N$ of carnivorous plants that have obtained none of their N from their prey, whilst $\delta^{15}N$ of insects can be used as a surrogate for carnivorous plants that have obtained all of their N from their prey. We believe that, for the purposes of this study, insects and noncarnivorous *Sphagnum* provide valid references for the likely $\delta^{15}N$ of carnivorous *Drosera* plants that have, respectively, obtained all, or none, of their N from captured prey, and that this approach provides a robust indication, within the limits of this research, of the contribution of captured insects to the N nutrition of the plants studied.

The data were evaluated using ANOVA and linear regression. A one-way ANOVA was used to test for differences in DIN between sites and to test for differences in $\delta^{13}C$ and $\delta^{15}N$ between the different plant/insect groups within each site. Analysis of differences between sites, and between plots within sites, of the proportion of N derived from prey, C : N ratio and $\delta^{13}C$ used a 'Randomized Block Design' with plots nested within site. Post-hoc comparisons used the Least Significant Difference (LSD) (0.05 significance level). All statistical analyses were carried out using Genstat version 6. Two values of the proportion of prey-derived N, which were effectively 0, were considered to be outliers and were excluded from analysis. This did not affect the significance of any of the tests, merely the strength of the significance.

Results

There were significant differences in $\delta^{15}N$ between the groups of plants and insects (Table 1). Specifically, at all sites, $\delta^{15}N$ of insects was significantly different from all other groups. Although there were no significant differences between *D. rotundifolia* and *D. intermedia*, both these groups were significantly different from both the *Sphagnum* spp. and the NCVPs. Therefore, it was considered appropriate to use the $\delta^{15}N$ values to calculate the percentage N originating from insects.

Table 1 Mean ($\bar{x}$) ± one Standard Error (SE), minimum and maximum values of $\delta^{15}N‰$ and $\delta^{13}C‰$ for two *Drosera* spp., one *Sphagnum* spp. sample and an aggregated sample of noncarnivorous vascular plants (NCVP) from three UK sites. *F*-value and significance of the ANOVA for within site interspecific comparisons are also given (ns = $P > 0.05$; * = $P < 0.01$; ** $P < 0.001$)

		D. rotundifolia				*D. intermedia*				*Sphagnum* spp.				NCVP				Insects				ANOVA
Site	Species	$\bar{x}$ ± SE	*n*	Min.	Max.	$\bar{x}$ ± SE	*n*	Min.	Max.	$\bar{x}$ ± SE	*n*	Min.	Max.	$\bar{x}$ ± SE	*n*	Min.	Max.	$\bar{x}$ ± SE	*n*	Min.	Max.	results
Lake District	$\delta^{15}N$	1.4 ± 0.2	24	−1.3	2.9		ND			−1.8 ± 0.5	5	−3.0	0.0	−1.1 ± 0.5	5	−2.8	0.1	5.0 ± 0.3	6	2.3	5.5	$F = 70.670^{**}$
	$\delta^{13}C$	−26.4 ± 0.1	25	−24.8	−27.6		ND			−26.2 ± 0.3	5	−25.5	−27.3	−26.3 ± 0.3	3	−25.8	−26.7	−25.2 ± 0.3	9	−24.6	−26.9	$F = 7.590^{**}$
New Forest	$\delta^{15}N$	2.6 ± 0.2	25	0.9	4.1	3.2 ± 0.3	10	1.4	3.7	−1.0 ± 0.3	4	−1.6	−0.1	−0.8 ± 0.3	3	−1.3	−0.2	5.5 ± 0.5	6	3.9	7.0	$F = 36.353^{*}$
	$\delta^{13}C$	−27.6 ± 0.2	25	−25.2	−29.0	−26.4 ± 0.4	10	−21.4	−28.0	−26.9 ± 0.7	5	−25.4	−29.2	−27.8 ± 0.2	4	−27.1	−28.2					$F = 4.040^{*}$
Rannoch Moor	$\delta^{15}N$	2.3 ± 0.2	24	−0.4	3.8	2.4 ± 0.2	10	1.8	4.4	−0.3 ± 0.3	5	−1.1	0.9	−1.7 ± 0.4	5	−2.6	−0.6	5.7 ± 1.7	3	2.3	8.1	$F = 39.895^{*}$
	$\delta^{13}C$	−27.0 ± 0.2	25	−24.8	−28.8	−26.8 ± 0.6	10	−21.6	−28.5	−26.9 ± 0.7	5	−24.7	−27	−26.6 ± 0.5	5	−25.5	−28.1	−25.1 ± 0.2	3	−25.5	−24.8	$F = 1.952^{ns}$

Between plant differences

For *D. rotundifolia*, the mean proportion of N obtained from prey for all samples was 50% (Fig. 1). ANOVA showed that overall there were significant differences in the proportion of N derived from prey between sites. Specifically, the mean proportion of N derived from prey at the Lake District and Rannoch Moor sites was significantly different from that at the New Forest site. There were also significant differences between plots within each site. There were no significant differences in the proportion of N derived from prey for *D. intermedia* at the two sites at which it was found (two tailed *T*-test, $P = 0.129$, d.f. = 17.815, $t = -1.592$). There were significant differences between sites in the C : N ratios of *D. rotundifolia* plants (Fig. 1). The C : N ratio of the plants at the New Forest site was significantly lower than that of plants at the Lake District and Rannoch Moor sites. There were no significant differences between plots within each site. Overall, there was a significant negative relationship between *D. rotundifolia* C : N ratio and the proportion of N the plants had obtained from their prey (Fig. 2). $\delta^{13}C$ varied significantly between sites, with lower values from the New Forest (Fig. 1), although the absolute differences were small. There was no significant effect of plot within each site on $\delta^{13}C$. The low variability in $\delta^{13}C$ is reflected in the differences between organism categories being significant only at the Lake District site (Table 1). All of the small sample of insects from the New Forest was required for nitrogen analysis and we were unable to determine of $\delta^{13}C$ of insects from that site.

Means (± SE) of total dissolved inorganic N (μgN l^{-1}) for New Forest, Lake District and Rannoch Moor were, respectively: 986 ± 44; 1250 ± 33; and 1090 ± 82 (Fig. 3). There were significant differences in the mean total dissolved inorganic N between the sites (1 way ANOVA, $F = 5.42$ $P = 0.021$, d.f. = 13). However, there were no significant relationships between total dissolved inorganic N and the proportion of N derived from prey (Pearson's correlation coefficient = −0.058, $P = 0.836$, $n = 15$) or between total dissolved inorganic N and C : N ratio (Pearson's correlation coefficient = 0.320, $P = 0.245$, $n = 15$).

Within plant differences

The proportion of N derived from prey differed significantly between the different plant parts (Fig. 4). The trend was for flowers to contain the highest proportion of prey derived N, followed by leaves, with roots having the lowest. Specifically, flowers had a significantly higher proportion of N derived from prey than did roots. There were also significant differences in the C : N ratio of the three separated plants parts (Fig. 4). The trend mirrored that of the proportion of N derived from prey, with the lowest C : N ratio in flowers followed by leaves, and roots having the highest C : N ratio. However, only the C : N ratio of roots was significantly

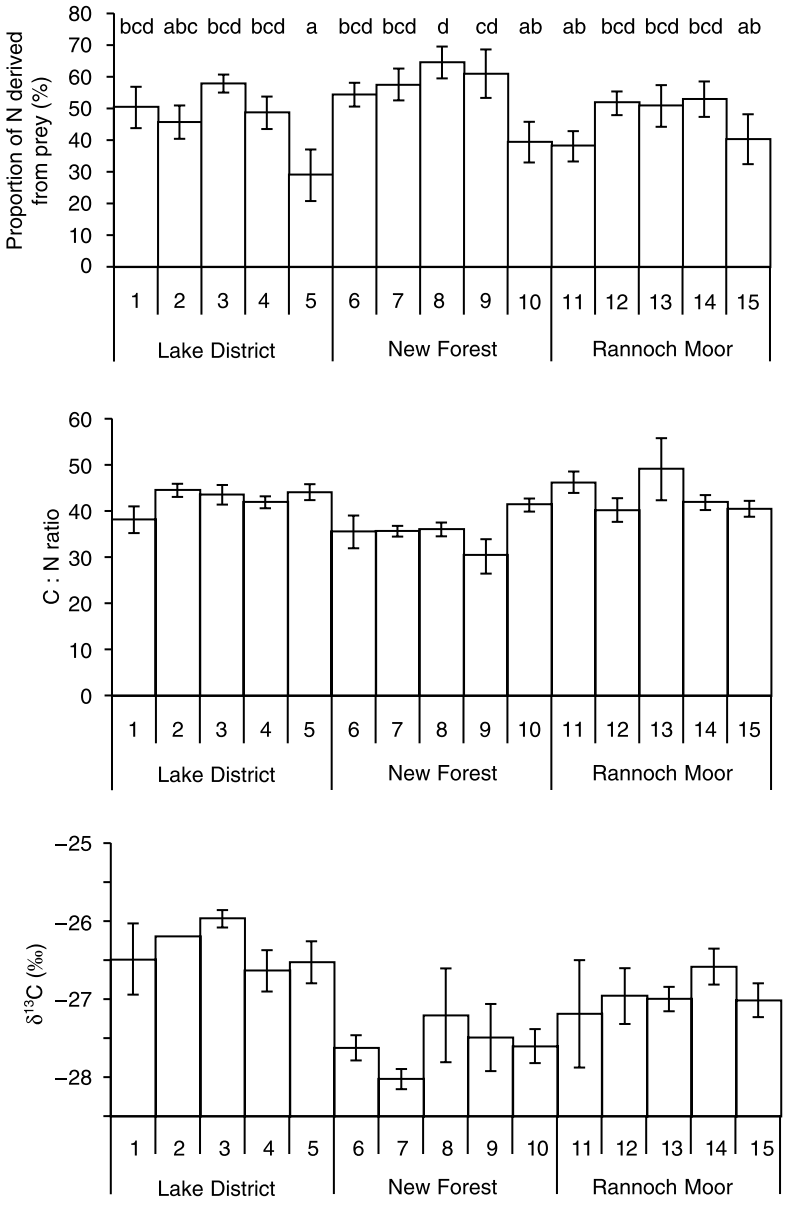

Fig. 1 Mean proportion of N derived from prey, C : N ratio and $\delta^{13}C$ (± 1 SE) for *Drosera rotundifolia* in sample plots at three UK sites. (ANOVA results: proportion of N derived from prey: site differences – $F = 5.97$, $P = 0.0259$; plot differences – $F = 6.97$, $P = 0.01$. C : N ratio: site differences – $F = 6.31$, $P = 0.0227$; plot differences – $F = 0.75$, $P = 0.587$. $\delta^{13}C$: site differences – $F = 26.56$, $P = 0.0003$; plot differences – $F = 1.03$, $P = 0.56$. For all analysis df = 2/4, $n = 5$ per plot). Plots for which the mean proportion of N derived from prey is significantly different from each other are signified by different letters (LSD; significance level = 0.05).

different from that of flowers and leaves, which were not significantly different from each other.

Discussion

Our results show that the *Drosera* plants studied obtained a substantial proportion (50%) of their N from captured prey, suggesting that captured insects are an important source of supplementary N. Our results are consistent with previous *in-situ* estimates of prey N contribution to total N content of 50% (Schulze *et al.*, 1991) and 53–68% (Moran *et al.*, 2001). This contrasts with some manipulative (Stewart & Nilsen, 1991) and *ex-situ* (Dore Swamy & Mohan Ram, 1971) experiments that found no benefit of carnivory, or concluded that the N obtained from captured prey was of no significance to the plants. The significant differences in mean proportion of N derived from prey in plots within sites may reflect variation in the insect capture rate. Karlsson *et al.* (1994) reported large between plant variations in prey capture rates. The significant between site differences in the proportion of N derived from prey mirrored the trend of DIN. This could indicate an increasing reliance on prey capture as available N decreases, in accordance with our first hypothesis. However, there was no significant correlation between DIN and the proportion of N derived from prey, which may reflect the rather limited range in DIN found in the study areas (848–1345 $\mu gN\ l^{-1}$). Hence the validity of our hypothesis requires further testing.

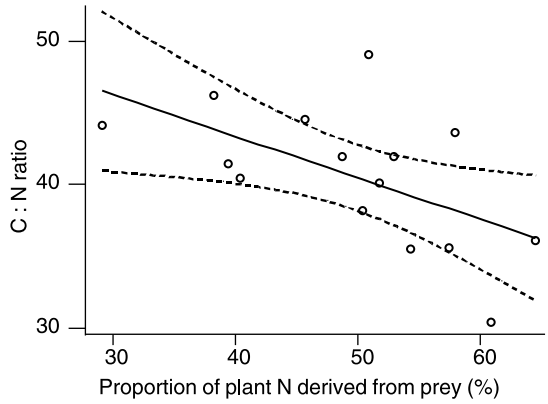

Fig. 2 Relation between proportion of N derived from prey (%) and C : N ratio for *Drosera rotundifolia* plants from three UK sites. Presented are means of five individual plants in each of 15 plots. There is a significant relationship between the proportion of N derived from prey and the C : N ratio of the plants. (Simple Linear Regression: $T = -2.51$, $P = 0.026$, $R^2 = 33\%$, regression equation is $a = 54.9 - 0.289b$ where a = C : N ratio and b = the proportion of N derived from prey). Solid line shows the fitted line, dashed line shows 95% confidence intervals.

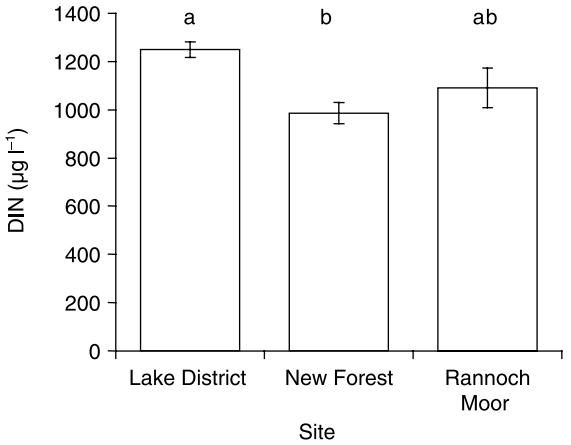

Fig. 3 Mean dissolved inorganic N (DIN) (± 1 SE) at three sites in the UK. (ANOVA, d.f. = 14; $F = 5.42$; $P = 0.021$; $n = 5$ per site). Sites for which the mean DIN is significantly different from each other are identifiable by different letters (LSD; significance level = 0.05).

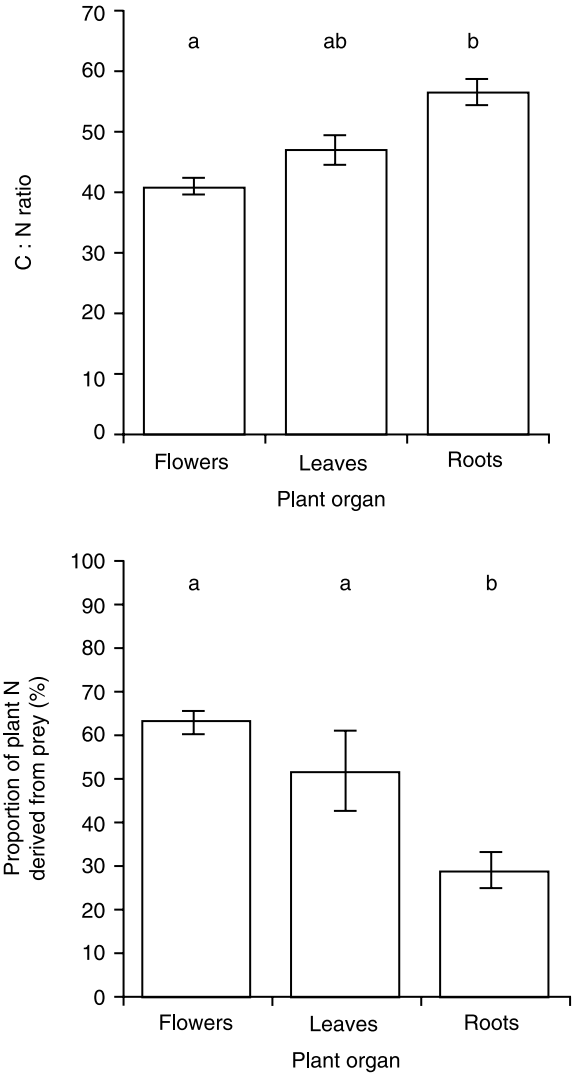

Fig. 4 C : N ratio (ANOVA, d.f. = 20, $F = 14.708$, $P < 0.001$) and proportion of N derived from prey (ANOVA, d.f. = 19, $F = 7.109$, $P = 0.006$) for separated plant parts of *Drosera rotundifolia*. Plant parts for which the mean values are significantly different from each other are indicated by different letters (LSD; significance level = 0.05). Values shown are means ± 1 SE.

The negative correlation between the proportion of N obtained from prey and the plant C : N ratio was consistent with our second hypothesis and provides evidence of a positive benefit of prey capture. This could be the result of increasing N stress as prey capture decreases, resulting in a higher C : N ratio, which would indicate that the plants are unable to mitigate decreased prey capture by increasing root N uptake, possibly due to an undeveloped root structure or a lack of available N in the substrate. This inference is consistent with the recent report from Adamec (2002) that prey capture by *Drosera* spp. actually stimulated root uptake of nutrients.

Although we also determined carbon isotope ratios, the lack of values for insects from the New Forest and the general low variability of the $\delta^{13}C$ results precludes analysis of the possible contribution of insect prey to the carbon content of the *Drosera*. Sensitivity analysis has shown that dietary mixing models in which the end member $\delta^{13}C$ values are indistinct will generate unacceptably large error variance in the contribution of the end members to a consumer (Vander Zanden & Rasmussen, 2001; Post, 2002). Therefore, in this paper we have focused on the contribution of carnivory to the nitrogen nutrition of *Drosera*.

The differences we found in the proportion of N derived from prey for different plant structures ($\delta^{15}N$ of the leaves higher than the rest of the plant) are comparable with those found by Schulze *et al.* (1991). These differences could be due

to discrimination in the allocation of insect-derived N, since Dixon *et al.* (1980) found that *D. erythrorhiza* did not allocate insect N equally throughout the plants. The differences could also be due to a delay in the distribution of leaf-derived N, or a result of the spatial proximity of the structures to the available N sources. Another feasible explanation is temporal changes in the availability of captured prey as a N source. When the roots are developing (before the first leaves have developed) the primary N source will initially be N stored from the previous year and then N taken up through the roots. As the first leaves develop, their initial N source will be root N uptake until the leaves are fully functional traps, after which N from captured prey will become progressively more important. By the time the flowers are developing there is a plentiful supply of insect-derived N from the rosette of fully developed leaf traps. As there are large differences in the calculated proportion of N derived from prey for different parts of the plant (i.e. the proportion of calculated N from prey in the flowers is approximately twice that for the roots) it is important that studies should use the entire plant when using the natural abundance stable isotope method for calculating the proportion of N derived from prey. The use of $\delta^{15}N$ for individual parts of plants rather than that from the entire plant, may result in erroneous calculations.

The proportion of N derived from prey was effectively 0% for two of the individual plants we analysed. These may just have been plants that happened to have caught no insects, although this seems improbable when all the other plants in the immediate vicinity were evidently successfully trapping insects and deriving half of their nitrogen in this way. Alternatively it is possible that these plants had failed to develop one of a number of the features required for the trapping and digestion of captured prey. For example, Dixon *et al.* (1980) described mutants of *Drosera erythrorhiza* that did not possess the stalked glands required to capture prey.

It is apparent from this study that prey capture is an important, but not an essential, component of the N nutrition of the *Drosera* plants studied. It appears that the plants benefit from N in the captured prey through a decreased C : N ratio, supporting our second initial hypothesis. However, it is not clear whether an increase in the proportion of plant N that is obtained from captured prey replaces or augments N obtained from root uptake. The small variation between our sites in dissolved inorganic nitrogen concentration in water did not allow us to evaluate our first initial hypothesis. Nevertheless, our findings extend previous work highlighting the value of the natural abundance stable isotope method as the only way of measuring the contribution of carnivory to the N budget of carnivorous plants *in-situ* and without manipulation.

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